Commentary

A sensible approach to targeting STAT3-mediated transcription

Neil E. Bhola, Daniel E. Johnson, Jennifer R. Grandis

Department of Otolaryngology, University of California San Francisco, San Francisco, CA, USA

Correspondence to: Jennifer R. Grandis. Department of Otolaryngology, University of California San Francisco, 550 16th Street, UCSF Box 0558, San Francisco, CA 94158, USA. Email: jennifer.grandis@ucsf.edu.

Submitted Sep 22, 2016. Accepted for publication Sep 25, 2016.
doi: 10.21037/atm.2016.10.51
View this article at: http://dx.doi.org/10.21037/atm.2016.10.51

Many cancers overexpress oncogenic proteins that drive robust tumorigenic signaling cascades which can be amenable to therapeutic targeting. However, inhibition of these proteins has been met with limited success to date, at least in part, due to the numerous feedback mechanisms driven by activation of alternative signaling pathways. To avoid the toxicities of targeting multiple receptors and oncogenic proteins, drug development focused on transcription factors is attractive. Transcription factors such as NF-κB, beta catenin and signal transducers and activators of transcription (STATs) are activated in the cytoplasm by numerous upstream signaling nodes before shuttling to the nucleus to drive transcription of mitogenic and antiapoptotic genes. Their ability to convey signaling from multiple oncogenic drivers and mediate gene expression makes them very promising therapeutic targets. An article published in November 2015 by Hong *et al.* in the journal *Science Translational Medicine* demonstrated that targeting the STAT family member STAT3 using an antisense oligonucleotide (ASO) AZD9150 resulted in potent and specific inhibition of STAT3 expression and suppression of lymphoma and lung cancer growth in preclinical models (1). They further observed antitumor activity with AZD9150 in a dose escalation study with 25 treatment-refractory patients.

STATs are a family of transcription factors that are phosphorylated by various upstream activators. Upon phosphorylation STATs enter the nucleus and form transcription complexes via their DNA-binding domains (DBD) (2,3). One STAT family member, STAT3, is constitutively active in numerous tumor models including lymphoma, lung cancer and head and neck squamous cell cancer (HNSCC). STAT3 drives the expression of genes such as *CCND1*, *MCL1*, *BCL2L1*, *BIRC5*, *IL6* and *MYC* (4) which have both tumor-intrinsic and extrinsic effects.

The STAT3 pathway can be targeted by inhibiting upstream JAK kinases (e.g., AZD1480), STAT3 dimerization (e.g., Stattic) (5) or STAT3-mediated DNA binding (STA-21, STAT3 Decoy) (6,7). However, JAK inhibitors have resulted in anemia and thrombocytopenia in clinical trials and JAK-independent mechanisms of STAT3 activation in cancer have been reported (8). Stattic has displayed promising potential in different cancer models but has also been shown to promote increased redox reactions that can trigger off-target effects (9). To specifically target STAT3, Hong *et al.* investigated the inhibition of STAT3 mRNA expression and transcriptional output using ASO technology. The authors modified their STAT3 ASO with constrained ethyl residues (cET) on either side of an 8–10 base phosphorothioate-modified deoxynucleotide, which improved stability and efficacy of targeting STAT3 mRNA both *in vitro* and *in vivo*. This was a critical finding since a major limitation of prior ASOs has been their poor cellular uptake. Importantly, the authors' cET modification to the STAT3 ASO permitted lipid-independent uptake into cell lines *in vitro*. Additionally, the modified STAT3 ASO did not inhibit STAT1 or STAT5 underscoring the specificity of the ASO. This is a key finding since another group of STAT3 inhibitors, the STAT3 decoy oligonucleotides, have been shown to inhibit both STAT3 and STAT1 (10,11). STAT1 plays an important role in negatively regulating cell growth (12,13); therefore, cross-inhibition of STAT1 by STAT3 decoys may diminish the antitumor effects of STAT3 blockade. However, STAT3 decoys with specificity to STAT3 alone are currently being developed (14).

Hong *et al.* observed that AZD9150 efficiently depleted STAT3 mRNA levels and abrogated the growth of lymphoma and NSCLC xenograft tumors *in vivo* when

used as a single agent. Notably, STAT3 activation has been shown to be induced by inhibitors of receptor tyrosine kinases or MEK in various oncogene-addicted solid tumor models (15-17). In one such study, RNAi-mediated inhibition of the STAT3 was sufficient to overcome erlotinib resistance (15). Furthermore, clinical testing of gefitinib and erlotinib in lung cancer demonstrated complete responses in patients with low STAT3 levels (RNA-seq) and increased recurrences in patients with high STAT3. Therefore, while AZD9150 may be potent against lymphomas as a monotherapy, combination of AZD9150 with other oncogenic targeting agents may be a promising therapeutic approach against solid tumors. This is concordant with the clinical efficacy observed in certain models by Hong *et al*., where AZD9150 resulted in tumor reduction in patients who failed prior therapeutic regimens.

While the findings by Hong and co-authors demonstrate the impact of STAT3 inhibition on tumor growth, their promising results with AZD9150 also highlights the potential therapeutic application of ASOs. In earlier studies, the application of methoxyethyl residues (MOEs) and locked nucleic acids (LNAs) proved to enhance the potency of ASOs; however, these additions were linked with increased toxicity when tested in preclinical and clinical settings. Combination of the structural elements of MOEs and LNAs led to the development of constrained MOEs (cMOE) and ethyl residues (cET) (18). Addition of the cMOE or cET modifications to ASOs improved potency and decreased susceptibility to nuclease digestion to a greater degree than the LNA or MOE modifications alone. As shown by Hong *et al*., AZD9150 displayed greater uptake and potency compared to the second-generation MOE-containing ASOs. The advances made in ASO chemistry can now be exploited for other difficult-to-target genes or driver mutations beyond STAT3. This chemistry can also be applied to the development of oligonucleotide decoy compounds that inhibit protein interaction functions and target specific cells. For example, Zhang *et al*. have shown that an immune cell-specific STAT3 decoy abrogated leukemia growth and immune checkpoint signaling in a mouse model (19). In this case, the STAT3 decoy was conjugated to a CpG decoy which targets the Toll-like receptor 9 (TLR9) found on immune cells. Their decoy only used phosphorothioate modifications to prevent nuclease digestion. Based on the findings with AZD9150, addition of the cET residues to the CpG-STAT3 decoy may further improve the efficacy and stability of this compound for clinical application.

Taken together, the results by Hong *et al*. demonstrate the potency of their ASO technology in achieving specific inhibition of STAT3 and the efficacy of their compound in promoting antitumor effects in preclinical and clinical settings. Since the patient cohort used for the dose escalation study was small, further investigations are needed to assess the efficacy and toxicity of AZD9150 in human cancer patients. Additionally, *in vitro* studies using RNAi molecules have been shown to activate distinct feedback mechanisms from those activated by pharmacological inhibitors (20). Evaluation of the potential feedback mechanisms activated by AZD9150 and similar ASOs also warrants further investigation in light of the promising preliminary findings reported by Hong *et al*. for AZD9150.

Acknowledgements

None.

Footnote

Provenance: This is a Guest Commentary commissioned by Section Editor Junhong Wang, MD, PhD (Department of Geriatric Medicine, the first affiliated hospital of Nanjing Medical University, Nanjing, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Hong D, Kurzrock R, Kim Y, *et al*. AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer. Sci Transl Med 2015;7:314ra185.

References

1. Hong D, Kurzrock R, Kim Y, et al. AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer. Sci Transl Med 2015;7:314ra185.
2. Yu H, Jove R. The STATs of cancer--new molecular targets come of age. Nat Rev Cancer 2004;4:97-105.
3. Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clin Cancer Res 2002;8:945-54.
4. Bromberg J. Stat proteins and oncogenesis. J Clin Invest 2002;109:1139-42.
5. Schust J, Sperl B, Hollis A, et al. Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. Chem

Biol 2006;13:1235-42.
6. Song H, Wang R, Wang S, et al. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. Proc Natl Acad Sci U S A 2005;102:4700-5.
7. Sen M, Thomas SM, Kim S, et al. First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. Cancer Discov 2012;2:694-705.
8. Singh R. Jak2-Independent Activation of Stat3 by Intracellular Angiotensin II in Human Mesangial Cells. J Signal Transduct 2011;2011:257862.
9. McMurray JS. A new small-molecule Stat3 inhibitor. Chem Biol 2006;13:1123-4.
10. Tadlaoui Hbibi A, Laguillier C, Souissi I, et al. Efficient killing of SW480 colon carcinoma cells by a signal transducer and activator of transcription (STAT) 3 hairpin decoy oligodeoxynucleotide--interference with interferon-gamma-STAT1-mediated killing. FEBS J 2009;276:2505-15.
11. Souissi I, Najjar I, Ah-Koon L, et al. A STAT3-decoy oligonucleotide induces cell death in a human colorectal carcinoma cell line by blocking nuclear transfer of STAT3 and STAT3-bound NF-κB. BMC Cell Biol 2011;12:14.
12. Stephanou A, Latchman DS. STAT-1: a novel regulator of apoptosis. Int J Exp Pathol 2003;84:239-44.
13. Najjar I, Fagard R. STAT1 and pathogens, not a friendly relationship. Biochimie 2010;92:425-44.
14. Souissi I, Ladam P, Cognet JA, et al. A STAT3-inhibitory hairpin decoy oligodeoxynucleotide discriminates between STAT1 and STAT3 and induces death in a human colon carcinoma cell line. Mol Cancer 2012;11:12.
15. Lee HJ, Zhuang G, Cao Y, et al. Drug resistance via feedback activation of Stat3 in oncogene-addicted cancer cells. Cancer Cell 2014;26:207-21.
16. Li G, Zhao L, Li W, et al. Feedback activation of STAT3 mediates trastuzumab resistance via upregulation of MUC1 and MUC4 expression. Oncotarget 2014;5:8317-29.
17. Zhao C, Li H, Lin HJ, et al. Feedback Activation of STAT3 as a Cancer Drug-Resistance Mechanism. Trends Pharmacol Sci 2016;37:47-61.
18. Seth PP, Vasquez G, Allerson CA, et al. Synthesis and biophysical evaluation of 2',4'-constrained 2'O-methoxyethyl and 2',4'-constrained 2'O-ethyl nucleic acid analogues. J Org Chem 2010;75:1569-81.
19. Zhang Q, Hossain DM, Duttagupta P, et al. Serum-resistant CpG-STAT3 decoy for targeting survival and immune checkpoint signaling in acute myeloid leukemia. Blood 2016;127:1687-700.
20. Weiss WA, Taylor SS, Shokat KM. Recognizing and exploiting differences between RNAi and small-molecule inhibitors. Nat Chem Biol 2007;3:739-44.

Cite this article as: Bhola NE, Johnson DE, Grandis JR. A sensible approach to targeting STAT3-mediated transcription. Ann Transl Med 2016;4(Suppl 1):S57. doi: 10.21037/atm.2016.10.51

